

FORM PTO-1390 (Modified) (REV 11-98)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 14014.0353U1
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371			U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5) 09/856544
INTERNATIONAL APPLICATION NO. PCT/US99/27817	INTERNATIONAL FILING DATE 23 November 1999	PRIORITY DATE CLAIMED 25 November 1998	
TITLE OF INVENTION ANTAGONISTS OF THE ALPHA E BETA 7 INTEGRIN AS THERAPEUTIC AGENTS FOR INFLAMMATORY DISEASES			
APPLICANT(S) FOR DO/EO/US LUDVIKSSON et al. <i>LUDVIKSSON BJORN R. et al.</i>			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
<ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1). 4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c) (2)) <ol style="list-style-type: none"> a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> has been transmitted by the International Bureau. c. <input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)). 7. <input checked="" type="checkbox"/> A copy of the International Search Report (PCT/ISA/210). 8. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3)) <ol style="list-style-type: none"> a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> have been transmitted by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input checked="" type="checkbox"/> have not been made and will not be made. 9. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 10. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). 11. <input checked="" type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409). 12. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)). 			
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ANTAGONISTS OF THE ALPHA E BETA 7 INTEGRIN AS THERAPEUTIC AGENTS FOR INFLAMMATORY DISEASES

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BACKGROUND OF THE INVENTION**FIELD OF THE INVENTION**

The present invention relates to a method of treating the inflammatory response of an autoimmune disease, allergic disease, graft-versus-host disease and
10 transplantation rejection. In particular, the present invention provides a method for treating and preventing an autoimmune disease, allergic disease, graft-versus-host disease and transplantation rejection by administering antagonists of the $\alpha^E\beta_7$ integrin. Further provided is a method of screening substances effective in reducing and preventing the inflammatory response of autoimmune disease, allergic disease, graft-
15 versus-host disease and transplantation rejection in an animal model.

BACKGROUND ART

The pathogenesis of autoimmune inflammatory reactions, both those occurring
20 in humans and those occurring in various murine models of autoimmunity may depend on the traffic of lymphocytes from sites of induction to sites of inflammation (1-4). Such traffic is now known to be mediated, at least in part, by an interaction between the integrin, $\alpha_4\beta_7$, on circulating lymphocytes and the addressin MAdCAM-1 on endothelial cells (5-10). The "homing" interactions governing the entry of cells into
25 tissues are accompanied by additional adhesion molecule-ligand interactions that ensure the retention of cells in the target tissue (11).

In recent studies, it has been shown that IL-2 $-/-$ mice do not develop significant colitis when maintained in a pathogen-free environment, but can be induced to develop
30 severe colitis rapidly and predictively following i.p. injection of TNP-substituted protein in complete Freund's adjuvant (12). Such induced colitis is a Th1 CD4

lymphocyte-driven inflammation that is similar to the spontaneous colitis developed in IL-2 $-/-$ mice maintained in a conventional environment (12-14).

5 The role of $\alpha^E\beta_7$ in this experimental model of chronic intestinal inflammation was analyzed in the IL-2 $-/-$ model. The present invention shows that administration of anti- $\alpha^E\beta_7$ prevents colonic inflammation and that anti- $\alpha^E\beta_7$ reverses pre-existing inflammation.

10 The present invention provides a novel method to prevent and treat inflammatory reactions in autoimmune disease, allergic disease, graft-versus-host disease and transplantation rejection by administering antagonists of $\alpha^E\beta_7$ integrin. The present invention further provides a method for screening for substances effective in their ability to inhibit the inflammatory effects of $\alpha^E\beta_7$ integrin.

15 SUMMARY OF THE INVENTION

The present invention provides a method of treating inflammation in a subject, comprising administering to the subject, an antagonist to $\alpha^E\beta_7$. The present invention further provides a method of preventing inflammation in a subject, comprising,
20 administering to the subject an antagonist to $\alpha^E\beta_7$.

Also provided is a method of screening for a substance effective in reducing the inflammatory effects of $\alpha^E\beta_7$, comprising administering the substance to an animal having an established inflammatory disease; assaying inflammatory tissue cells from
25 the animal for an amount of secretion of proinflammatory cytokines, inflammatory cytokines or chemokines, whereby a decrease in the amount of proinflammatory cytokines, inflammatory cytokines or chemokines secreted by the inflammatory tissue cells of the animal as compared to the amount of proinflammatory cytokines, inflammatory cytokines or chemokines secreted by inflammatory tissue cells of a
30 control animal having an inflammatory disease and without having the substance administered indicates the substance is effective in reducing the inflammatory effect of

$\alpha E\beta 7$. This method can further comprise the step of evaluating the effect of the antagonist of $\alpha E\beta 7$ in reducing the adhesion/retention/influx of $\alpha E\beta 7$ + inflammatory cells into the inflammatory tissue.

- 5 Additionally, the present invention provides a method of screening for a substance effective in preventing the inflammatory effects of $\alpha^E\beta_7$, comprising administering the substance to an animal susceptible to an inflammatory disease; subjecting the animal to treatment that will induce an inflammatory response; assaying inflammatory tissue cells from the animal for an amount of secretion of
- 10 proinflammatory cytokines, inflammatory cytokines or chemokines, whereby a lack of increase in the amount of proinflammatory cytokines, inflammatory cytokines or chemokines secreted by the inflammatory tissue cells of the animal as compared to an increase in the amount of proinflammatory cytokines, inflammatory cytokines or chemokines secreted by inflammatory tissue cells of a control animal having an
- 15 inflammatory disease and without having the substance administered indicates the substance is effective in preventing the inflammatory disease by inhibiting the inflammatory effect of $\alpha^E\beta_7$. This method can further comprise the step of evaluating the effect of the antagonist of $\alpha E\beta 7$ in reducing the adhesion/retention/influx of $\alpha E\beta 7$ + inflammatory cells into the inflammatory tissue.

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BRIEF DESCRIPTION OF THE DRAWINGS

- Figure 1. Administration of anti- $\alpha^E\beta_7$ mAb prevents and ameliorates TNP-OVA induced colitis in IL-2-/- mice. Photomicrographs from hematoxylin/eosin-stained
- 25 colonic tissue. A, normal colonic tissue from mouse that was injected with PBS and rat IgG2a. B, significant mononuclear cell inflammation seen in colonic mucosa of a IL-2-/- mouse immunized with TNP-OVA in CFA and co-injected with rat IgG2a. C and D, near-normal appearance of the colonic mucosa following either preventive or treatment regimen of anti- $\alpha^E\beta_7$ mAb in mice injected with TNP-OVA in CFA, respectively. 100X
- 30 magnification. Results represent data obtained from 3-4 mice in each group.

Figure 2. Decreased number of CD4⁺ LPL following treatment with anti- $\alpha^E\beta_7$ mAb. LPL were isolated, counted and analyzed for CD4⁺ and CD8⁺ phenotype by flow cytometry (see Examples). Total no. of LPL was analyzed from IL-2^{-/-} mice that were injected with PBS + Rat IgG2a (n=3; white bars), TNP-OVA in CFA + Rat IgG2a (n=4; black bars) and TNP-OVA in CFA + anti- $\alpha^E\beta_7$ (n=4; dotted bars). *p < 0.01.

Figure 3. Total number of CD4⁺ and CD8⁺ IEL following treatment with anti- $\alpha^E\beta_7$ mAb. IEL were isolated, counted and analyzed for CD4⁺ and CD8⁺ phenotype by flow cytometry (see Examples). Total no. of IEL was analyzed from IL-2^{-/-} mice that were injected with PBS + Rat IgG2a (n=3; white bars), TNP-OVA in CFA + Rat IgG2a (n=4; black bars) and TNP-OVA in CFA + anti- $\alpha^E\beta_7$ (n=4; dotted bars).

Figure 4. Increased number of CD4⁺ lymphocytes within the spleen following treatment with $\alpha^E\beta_7$ mAb. Splenic lymphocytes were isolated, counted and analyzed for CD4⁺ and CD8⁺ phenotype by flow cytometry (see Examples). Total no. of splenic lymphocytes was analyzed from IL-2^{-/-} mice that were injected with PBS + Rat IgG2a (n=3; white bars), TNP-OVA in CFA + Rat IgG2a (n=4; black bars) and TNP-OVA in CFA + anti- $\alpha^E\beta_7$ (n=4; dotted bars). *p < 0.01.

Figure 5. Increased IFN- γ production of splenic lymphocytes following treatment with anti- $\alpha^E\beta_7$ mAb. Splenic lymphocytes were isolated from IL-2^{-/-} mice that were injected with PBS + Rat IgG2a (n=3; white bars), TNP-OVA in CFA + Rat IgG2a (n=4; black bars) and TNP-OVA in CFA + anti- $\alpha^E\beta_7$ (n=4; dotted bars). Splenic lymphocytes were then stimulated in vitro with anti-CD3 + anti-CD28 and IFN- γ production was analyzed by ELISA in cell culture supernatants after 48 hours (see Examples). *p < 0.01.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the invention and the
5 Example included therein.

Before the present compounds and methods are disclosed and described, it is to be understood that this invention is not limited to specific proteins or specific methods. It is also to be understood that the terminology used herein is for the purpose of
10 describing particular embodiments only and is not intended to be limiting.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

15 The present invention provides a method of treating inflammation in a subject, comprising, administering to the subject an antagonist to $\alpha^E\beta_7$. The present invention further provides a method of preventing inflammation in a subject, comprising, administering to the subject an antagonist to $\alpha^E\beta_7$. The inflammatory response can be
20 associated with autoimmune disease, allergic diseases, graft vs. host disease, or transplantation rejection, to name a few.

The present invention also provides a method for preventing the activation, influx and retention of inflammatory cells by administering an antagonist of the $\alpha^E\beta_7$
25 integrin and its known and future ligands in a subject having an autoimmune disease, allergic disease GvH disease or transplant rejection in amounts sufficient to treat or prevent the inflammatory disease.

In the present invention, the subject can be any mammal, preferably human,
30 and can include but is not limited to mouse, rat, guinea pig, hamster, rabbit, cat, dog, goat, monkey, horse and chimpanzee.

As used herein, "treating" describes an improvement in the patient's clinical state. The improvement may range from reduction of the inflammatory response to complete amelioration of the inflammatory disease.

5 As used herein, "autoimmune disease" describes a disease state or syndrome whereby a subject's body produces a dysfunctional immune response against the subject's own body components, with adverse effects. This may include production of B cells which produce antibodies with specificity for all antigens, allergens or major histocompatibility (MHC) antigens, or it may include production of T cells bearing
10 receptors that recognize self-components and produce cytokines that cause inflammation. Examples of autoimmune diseases include, but are not limited to, ulcerative colitis, Crohn's disease, multiple sclerosis, rheumatoid arthritis, diabetes mellitus, pernicious anemia, autoimmune gastritis, psoriasis, Bechet's disease, Wegener's granulomatosis, Sarcoidosis, autoimmune thyroiditis, autoimmune
15 oophoritis, bullous pemphigoid, pemphigus, polyendocrinopathies, Still's disease, Lambert-Eaton myasthenia syndrome, myasthenia gravis, Goodpasture's syndrome, autoimmune orchitis, autoimmune uveitis, systemic lupus erythematosus, Sjogren's Syndrome and ankylosing spondylitis (15-22).

20 As used herein, "allergic disease" describes a disease state or syndrome whereby the body produces a dysfunctional immune response composed of Immunoglobulin E (IgE) antibodies to environmental antigens and which evoke allergic symptoms. Examples of allergic disease include, but are not limited to, asthma, ragweed pollen hayfever, allergy to food substances, atopic eczema, hypersensitivity
25 pneumonitis, Farmers lung, Hyper eosinophilic syndromes and allergic reactions (23-25).

As used herein, "graft-versus-host" (GvH) disease describes a disease state or syndrome whereby an immune response is initiated by grafted cells and is directed
30 against the subject's body with adverse effects. Examples of GvH disease include, but

are not limited to, acute and chronic GvH disease following bone marrow transplant (26).

Transplantation rejection describes a disease state or syndrome whereby the transplant recipient's body produces an immune response against the engrafted tissue, resulting in rejection. Transplantation rejection can occur, for example, with kidney, heart, lung or liver transplants as well as with any other transplanted tissue (27,32).

The antagonist of $\alpha^E\beta_7$ can be an antibody, either polyclonal or monoclonal, that specifically binds with $\alpha^E\beta_7$ or a ligand that binds to $\alpha^E\beta_7$. Anti-idiotypic antibodies and affinity matured antibodies are also considered. Other antagonists of $\alpha^E\beta_7$ can include, but are not limited to inhibitors of $\alpha^E\beta_7$ production, inhibitors of $\alpha^E\beta_7$ expression, and molecules designed to block $\alpha^E\beta_7$ activation or binding. The antagonist can be a whole protein or a fragment of a protein that inhibits the activity, production, expression, activation and binding of $\alpha^E\beta_7$. For example, a molecule that blocks $\alpha^E\beta_7$ binding can be a molecule that binds to the cellular receptor for $\alpha^E\beta_7$. Crystal structures of $\alpha^E\beta_7$ and its ligand, E-cadherin, may be utilized to design molecules that may disrupt the $\alpha^E\beta_7$ -ligand interaction.

The phrase "specifically binds" with the $\alpha^E\beta_7$ refers to a binding reaction which is determinative of the presence of the $\alpha^E\beta_7$ in a heterogeneous population of proteins and other biologics. Thus, under designated conditions, the specified antibodies bound to a particular protein (e.g. $\alpha^E\beta_7$ or the cellular receptor for $\alpha^E\beta_7$) do not bind in a significant amount to other proteins present in the subject. Selective binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. A variety of immunoassay formats may be used to select antibodies that selectively bind with a particular protein (e.g. $\alpha^E\beta_7$ or the cellular receptor for $\alpha^E\beta_7$). For example, solid-phase ELISA immunoassays are routinely used to select antibodies selectively immunoreactive with a protein. See *Harlow and Lane* "Antibodies, A Laboratory Manual" Cold Spring Harbor Publications, New York,

(1988), for a description of immunoassay formats and conditions that could be used to determine selective binding.

The antibodies of this invention can be from any source. However, to reduce the immunogenicity of the immunoglobulins themselves, antibodies are preferably of human origin or are antibodies generated in other species and "humanized" for administration in humans as described in the Example. The antibodies of this invention can be fragments which retain the ability to bind their specific antigens. For example, fragments of antibodies which maintain $\alpha^E\beta_7$ binding activity, as well as fragments of $\alpha^E\beta_7$ which maintain $\alpha^E\beta_7$ binding activity (e.g. homodimer formation) but which function to inhibit the receptor-ligand binding interaction and activity of $\alpha^E\beta_7$, are included within the meaning of the term "antibody". Such antibodies and fragments can be made by techniques known in the art and screened for specificity and activity according to the methods set forth in the Example herein. For example, general methods for producing antibodies can be found in *Harlow and Lane* "Antibodies, A Laboratory Manual" Cold Spring Harbor Publications, New York, (1988).

In the present invention, the antagonist of $\alpha^E\beta_7$ can be orally or parenterally administered in a carrier pharmaceutically acceptable to human subjects. The antagonist of $\alpha^E\beta_7$ can also be administered in an enema, or as an inhalant, nasal spray or eye drops. Suitable carriers for oral or inhaled administration of the antagonist of $\alpha^E\beta_7$ can include one or more of the carriers pharmaceutically acceptable to human subjects. Suitable carriers for oral administration of the antigen include one or more substances which may also act as a flavoring agents, lubricants, suspending agents, or as protectants. Suitable solid carriers include calcium phosphate, calcium carbonate, magnesium stearate, sugars, starch, gelatin, cellulose, carboxypolymethylene, or cyclodextrans. Suitable liquid carriers may be water, pyrogen free saline, pharmaceutically accepted oils, or a mixture of any of these. The liquid can also contain other suitable pharmaceutical addition such as buffers, preservatives, flavoring agents, viscosity or osmo-regulators, stabilizers or suspending agents. Examples of suitable liquid carriers include water with or without various additives, including

carboxypolymethylene as a pH-regulated gel. The antibody may be contained in enteric coated capsules that release the antigen into the intestine to avoid gastric breakdown.

For parenteral administration of the antagonist, a sterile solution or suspension is prepared in saline that may contain additives, such as ethyl oleate or isopropyl

- 5 myristate, and can be injected for example, into subcutaneous or intramuscular tissues, as well as intravenously. The antagonist of $\alpha^E\beta_7$ or its ligand may be contained in enteric coated capsules that release the inhibitor into the intestine to avoid gastric breakdown.

- 10 Alternatively, the antagonist of $\alpha^E\beta_7$ may be microencapsulated with either a natural or a synthetic polymer into microparticles 4-8 μm in diameter, which target intestinal lymphoid tissues and produce a sustained release of inhibitor for up to four weeks.

- 15 In addition to the oral administration of antigen to a human subject, antagonist to $\alpha^E\beta_7$ or its ligand, in soluble form, would typically be administered parenterally in a single or multiple dosages of between 0.1 mg and 100 mg/kg of body weight, with preferred dosage range of 1-50 mg/kg and most preferred dosage of 2-10 mg/kg. Subjects can be given antibodies to $\alpha^E\beta_7$ as a single injection approximately weekly for
- 20 between one and 52 weeks. For oral administration, 500 mg to 1000 mg of antibodies to $\alpha^E\beta_7$ can be given PO. For administration of antibodies to $\alpha^E\beta_7$ in particulate form, 500 mg to 1000 mg can be microencapsulated as described for slow release over a four to eight week period. The treatment can be reinitiated at any time, particularly in the event of a recurrence. Depending on the particular inflammatory disease and the
- 25 patient's condition, a maintenance dosage may be administered to prevent reoccurrence. This dosage can be administered every other week to monthly depending on the nature of the inflammation which may vary from an acute condition to a maintenance state.

- The amount of antagonist of $\alpha^E\beta_7$ administered will vary among individuals
- 30 based on age, size, weight, condition, etc. One skilled in the art will realize that

dosages are best optimized by the practicing physician and methods for determining dosage are described, for example in Remington's Pharmaceutical Science.

The efficacy of administration of a particular dose of an antagonist of $\alpha^E\beta_7$ in
5 treating an autoimmune disease, allergic disease, GvH disease or transplantation
rejection in a subject diagnosed as having an autoimmune disease, allergic disease,
GvH disease or transplantation can be determined by standard methods of evaluation of
the particular signs, symptoms and objective laboratory tests for a particular disease, as
known in the art. For example, if 1) a subject's frequency or severity of recurrences is
10 shown to be improved, 2) the progression of the disease is shown to be stabilized, or 3)
the need for use of other immunosuppressive medications is lessened, based on a
comparison with an appropriate control group, and knowledge of the normal
progression of disease in the general population or the particular individual, then a
particular treatment will be considered efficacious.

15
The efficacy of an antagonist of $\alpha^E\beta_7$ in preventing an autoimmune disease,
allergic disease, GvH disease or transplantation rejection in a subject not known to have
an autoimmune disease, allergic disease, GvH disease or transplantation rejection can
be determined by evaluating standard signs, symptoms and objective laboratory tests,
20 as would be known to one of skill in the art, over time. This time interval may be large,
with respect to the development of an autoimmune disease or allergic disease
(years/decades) or short (weeks/months) with respect to the development of GvH
disease or transplantation rejection. The determination of who would be at risk for the
development of an autoimmune disease or allergic disease would be made based on
25 current knowledge of the known risk factors for a particular disease familiar to a
clinician in this field, such as a particularly strong family history of disease. For GvH
disease and transplantation rejection, patients undergoing transplant procedures would
be considered at risk for the development of these diseases.

30 The invention further provides a method for treating inflammation comprising
administering an antagonist to $\alpha^E\beta_7$ and another therapeutic agent. The invention also

provides a method for preventing inflammation comprising administering an antagonist to $\alpha^E\beta_7$ and another therapeutic agent.

Other therapeutic agents may include, but are not limited to, antibodies, such as
5 anti- $\alpha^4\beta_7$ Mab, cytokines, immunosuppressive agents or immunomodulatory agents.

Examples of immunosuppressive medication include, but are not limited to, corticosteroids, Mofetil, cytotoxic drugs (Azathioprine, Chlorambucil, Cyclophosphamide, Methotrexate, Cyclosporin, FK-506, 6-MP), and Thalidomide (27-
10 28). An example of combination therapy with corticosteroids would include the administration of an inhibitor to $\alpha^E\beta_7$ or its ligand with the combination of corticosteroids in either a low dose 2.5-20 mg/daily or a high dose 0.5-2 mg/Kg/day regimen, orally or intravenously. This would also include the use of corticosteroids in any other carrier such as an inhalants or an enema. An example of combination therapy
15 with azathioprine would be an oral administration of 1.25-2.5 mg/Kg/day. An example of combination therapy with cyclophosphamide would be an oral dose of 1-3 mg/kg/day or an intravenous dose of 10 –20 mg/Kg/every 1-3 months. An example of combination therapy with Methotrexate would be an oral regimen of 2.5 – 25 mg weekly.

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Examples of immunomodulatory agents include, but are not limited to, IVIG, administration of antisera against lymphocyte membrane antigens (i.e. antithymocyte serum (ATS), antithymocyte globulin (ATG), antilymphocyte serum (ALS), antilymphocyte globulin (ALG), anti-CD3, anti-CD4, anti-CD8)), anti-TNF α , anti-IL-
25 12, anti-IFN γ , antisense STAT4 oligonucleotides, anti-ICAM1, antisense ICAM-1 oligonucleotides, anti-CD40L, anti-CD25 (anti-Tag), and IL-10 (29-30).

Immunomodulators can be administered both to treat an acute episode of disease or to maintain the patient.

30 The efficacy of administration of a combination therapy as described herein in treating an autoimmune disease, allergic disease, GvH disease or transplantation

rejection in a subject diagnosed as having an autoimmune disease, allergic disease, GvH disease or transplantation can be determined by standard methods of evaluation of the particular signs, symptoms and objective laboratory tests for a particular disease, as known in the art. For example, 1) a subject's frequency or severity of recurrences is shown to be improved, 2) the progression of the disease is shown to be stabilized, or 3) the need for use of other immunosuppressive medications is lessened, based on a comparison with an appropriate control group and knowledge of the normal progression of disease in the general population or the particular individual, then a particular treatment will be considered efficacious.

10

The efficacy of combination therapy with any antibodies, immunosuppressive medications or immunomodulatory agents and an antagonist of $\alpha^E\beta_7$ in preventing an autoimmune disease, allergic disease, GvH disease or transplantation rejection in a subject not known to have an autoimmune disease, allergic disease, GvH disease or transplantation rejection can be determined by evaluating standard signs, symptoms and objective laboratory tests, as would be known to one of skill in the art, over time. This time interval may be large, with respect to the development of an autoimmune disease or allergic disease (years/decades) or short (weeks/months) with respect to the development of GvH disease or transplantation rejection. The determination of who would be at risk for the development of an autoimmune disease or allergic disease would be made based on current knowledge of the known risk factors for a particular disease familiar to a clinician in this field, such as a particularly strong family history of disease. For GvH disease and transplantation rejection, patients undergoing transplant procedures would be considered at risk for the development of these diseases.

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The present invention further provides compositions comprising an $\alpha^E\beta_7$ antibody and a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject, along with the antagonist without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The

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carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be recognized by one skilled in the art.

- 5 Also provided are compositions comprising an $\alpha^E\beta_7$ antagonist and a pharmaceutically acceptable carrier, wherein the antagonist is not an $\alpha^E\beta_7$ antibody.

Also provided are compositions comprising an $\alpha E\beta 7$ antibody and a pharmaceutically acceptable carrier, wherein the carrier is not hybridoma supernatant.

- 10 In this invention, a pharmaceutically acceptable carrier would not be ascites fluid.

In another embodiment, the present invention provides an $\alpha^E\beta_7$ antibody, a second anti-inflammatory agent and a pharmaceutically acceptable carrier.

- 15 In another embodiment, the present invention provides a method of screening for a substance effective in reducing the inflammatory effects of $\alpha^E\beta_7$ comprising administering the substance to an animal having an established inflammatory disease; assaying inflammatory tissue cells from the animal for an amount of secretion of proinflammatory cytokines, inflammatory cytokines or chemokines, whereby a
20 decrease in the amount of proinflammatory cytokines, inflammatory cytokines or chemokines secreted by the inflammatory tissue cells of the animal as compared to the amount of proinflammatory cytokines, inflammatory cytokines or chemokines secreted by inflammatory tissue cells of a control animal having an inflammatory disease and without having the substance administered indicates the substance is effective in
25 reducing the inflammatory effect of $\alpha E\beta 7$. The method can further comprise the step of evaluating the effect of the antagonist of $\alpha E\beta 7$ in reducing the adhesion/retention/influx of $\alpha E\beta 7^+$ inflammatory cells into the inflammatory tissue. A substance effective in reducing the inflammatory response of an established inflammatory disease is one that reduces or reverses the histological and clinical
30 manifestations of the inflammatory disease, as would be recognized by someone skilled in the art. Furthermore, an independent method comprising determining the effect of a

substance on the adhesion, retention, or influx of $\alpha E\beta 7$ inflammatory cells can be used to identify antagonists of $\alpha E\beta 7$.

In assaying inflammatory tissue cells, several inflammatory cytokines and
5 chemokines can be assayed. These include, but are not limited to IFN- γ , TNF- α , IL-4, IL-5, or IL-12, depending on the inflammatory disease in question.

Also provided by the present invention is a method of screening for a substance effective in preventing the inflammatory effects of $\alpha^E\beta_7$, comprising administering the
10 substance to an animal susceptible to an inflammatory disease; subjecting the animal to treatment that will induce an inflammatory response; assaying inflammatory tissue cells from the animal for an amount of secretion of proinflammatory cytokines, inflammatory cytokines or chemokines, whereby a lack of increase in the amount of proinflammatory cytokines, inflammatory cytokines or chemokines secreted by the
15 inflammatory tissue cells of the animal as compared to an increase in the amount of proinflammatory cytokines, inflammatory cytokines or chemokines secreted by inflammatory tissue cells of a control animal having an inflammatory disease and without having the substance administered indicates the substance is effective in preventing the inflammatory disease by inhibiting the inflammatory effect of $\alpha^E\beta_7$. The
20 method can further comprise the step of evaluating the effect of the antagonist of $\alpha E\beta 7$ in reducing the adhesion/retention/influx of $\alpha E\beta 7$ + inflammatory cells into the inflammatory tissue. Furthermore, an independent method comprising determining the effect of a substance on the adhesion, retention, or influx of $\alpha E\beta 7$ inflammatory cells can be used to identify antagonists of $\alpha E\beta 7$.

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In the present screening methods, the animal in which the inflammation is produced can be any mammal and can include but is not limited to mouse, rat, guinea pig, hamster, rabbit, cat, dog, goat, monkey, and chimpanzee. The inflammation can be produced in the animal by any method known in the art. For example, the
30 inflammation can be produced by introducing into the animal an effective amount of a hapten reagent. The hapten reagent can be, but is not limited to a 2,4,6-trinitrophenol

conjugate of keyhole limpet hemocyanin, 2,4,6-trinitrobenzene sulfonic acid, 2,4,6-dinitrochlorobenzene and other trinitrophenylamine compounds.

The present invention also provides in vitro screening methods for antagonists to $\alpha^E\beta_7$. In vitro cellular adhesion studies may be used to analyze the effect of chemically engineered compounds to block the binding of $\alpha^E\beta_7$ to its ligands or to analyze its ability to reduce cellular activation and pro-and inflammatory cytokine production (29,31,33). Examples of markers for cellular activation include, but are not limited to, CD25, CD45, CD69 and L-selectin.

Also provided are methods for assaying the amount of pro and inflammatory cytokines, and chemokine secretion from inflammatory tissue obtained from lamina propria, lymph nodes, spleen, liver, skin, lungs, joints, CNS and other tissues involved in inflammatory. These methods include, but are not limited to, flow cytometry, ELISA, PCR, reverse-transcriptase-polymerase chain reaction and ELISPOT, Northern blots, Southern blots, and Western blots (31). A reduced amount of cytokine or chemokine indicates the presence of a substance that may be acting at the level of $\alpha^E\beta_7$.

The present invention is more particularly described in the following examples which are intended as illustrative only since numerous modifications and variations therein will be apparent to those skilled in the art.

Examples

Mice

Mice rendered IL-2 deficient via gene targeting (34) were graciously donated by W. Paul (NIAID, NIH). Colonies of mice were generated by breeding heterozygous parents, and 4 wk after birth, homozygous mice were identified by PCR analysis of DNA isolated from digested tail snips as described (12). Mice were housed in a specific pathogen free (SPF) animal facility on the Bethesda campus of the National Institutes of Health. Prior to study, all mice were handled with gloves under a class II hood, fed sterile food and water ad libitum, and maintained in sterile microisolators that

were changed weekly. Selected tissues of various mice were collected for histopathology and endotape testing; cecum and colons were collected for parasitology. Such tissues tested negative for mouse adenoviruses, carbocillus, ectromelia, EDIM (epizootic diarrhea of infant mice), GDVII (George disease, VII),

- 5 lymphocytomegalovirus, mouse hepatitis virus, *Mycoplasma*, mouse pneumonia virus, reovirus, sendavirus, mouse cytomegalovirus, and parvovirus. Mice of both sexes aged 4-8 wk were used.

Production of humanized mouse antibodies to $\alpha^E\beta_7$.

- 10 Rodent monoclonal or polyclonal antibodies can be modified according to the protocols set forth in Junghans et.al. (36), Brown et.al. (37), Kettleborough et.al. (38). Specifically, rodent antibodies can be modified for human administration by constructing, through recombinant DNA protocols known to one of skill in the art, a chimeric rodent-human antibody composed of rodent variable regions and human
- 15 heavy and light chain constant regions. Another approach to humanizing rodent antibodies is to graft rodent complementary-determining regions (CDRs) from the rodent variable regions into human variable regions. By using either of these approaches, rodent antibodies can be humanized for administration into human subjects.

20

Immunization and treatment of mice

- Mice (4-8 weeks) were immunized once with 2,4,6-trinitrophenol (TNP) conjugate of keyhole limpet hemocyanin (KLH) in complete Freund's adjuvant. The conjugate was prepared as follows: 20 mg of OVA (Pierce Chemical Co., Rockford, IL) reconstituted in 2.0 ml distilled water was added to 2.0 ml of potassium borate buffer
- 25 (pH 9.0-9.3), then mixed with 3.0 ml of 1 mg/ml solution of TNBS (gift from Dr. J. Inman, NIAID, NIH) in distilled water and 8 μ l of 1 M Na_2CO_3 added; the mixture was allowed to sit in the dark for 8 h at room temperature and dialyzed 12 h in 1X PBS, after which the concentration of the conjugate was determined via BCA
- 30 protein assay reagent (Pierce Chemical Co.). Mice were injected i.p. with 100 μ g TNP-OVA emulsified in Freund's complete adjuvant (Sigma ImmunoChemicals,

St. Louis, MO). For control, mice were injected with PBS emulsified in Freund's complete adjuvant.

To determine the role of $\alpha_4\beta_7$ and $\alpha^E\beta_7$ in the development of colitis in IL-2^{-/-} mice, the latter were administered 0.5 mg of anti- $\alpha_4\beta_7$ (Rat IgG2a mAb, Clone DATK32, Pharmingen), anti- $\alpha^E\beta_7$ (Rat IgG2a mAb, Clone M290, Pharmingen) mAb or Rat-IgG2a κ control Ab (Pharmingen) i.p. 1 h prior to TNP-OVA immunization (day 0) and the same amount of mAbs were readministered on day 2, 4 and 6 until mice were sacrificed. In some studies, groups of mice that had previously been immunized with TNP-OVA and had developed colitis were injected with 0.5 mg of anti- $\alpha^E\beta_7$ every other day times 3 and evaluated on the 7th day from the first anti- $\alpha^E\beta_7$ mAb injection. FACS analysis of double-stained of LP cells with anti- $\alpha_4\beta_7$ and anti- $\alpha^E\beta_7$ mAb revealed no cross-reactivity between the anti- $\alpha_4\beta_7$ and anti- $\alpha^E\beta_7$.

15 *Grading of histology changes*

Colons were dissected from immunized mice and processed for the preparation of frozen sections. Briefly, small sections, approximately 3-5 mm, were cut from each tissue and submerged in dry ice-cooled O.C.T. Embedding Compound (Tissue Tek; Miles Inc., Elkhart, IN). After complete solidification of the embedding media, samples were stored at -80°C.

Tissues were removed at indicated time points, cryopreserved, fixed, and embedded in paraffin. The embedded tissues were sectioned and stained with hematoxylin and eosin (American Histo Labs, Rockville, MD). The degree of inflammation of the colon was graded semiquantitatively from 0 to 4 (0, no signs of inflammation; 1, very low level and 2, low level of mononuclear cell infiltration; 3, high level of mononuclear cell infiltration, high vascular density, thickening of the colon wall; 4, transmural infiltration, loss of goblet cells, high vascular density, thickening of the colon wall).

IEL and Lamina propria cell isolation

Preparation of IEL and LP cell has been previously described (12). Briefly, detritus was squeezed out of the colon lumen with forceps, the tissue minced with sterile scissors, and the pieces washed with HBSS over a 100 mm nylon mesh bowl to
5 remove remaining detritus and metabolites. IEL were liberated by incubating the cut pieces in 25 ml of warm (37°C) HBSS media without Ca/Mg (Biofluids, Inc., Rockville, MD) supplemented with 25 mM HEPES buffer (NIH Media Unit) and 10% FCS (BioWhittaker, Walkersville, MD) containing 0.1M EDTA for 20 min at 37°C. Filtered cells were enriched for lymphocytes by Percoll-gradient centrifugation. For
10 Colonic lamina propria lymphocytes (LPL) isolation, the remaining pieces of colon were washed over nylon mesh, resuspended in RPMI 1640 media (BioWhittaker) augmented with 25 mM HEPES buffer, 10% FCS, 400 U/ml DNase (Boehringer Mannheim Biochemical, Indianapolis, IN), 400 U/ml collagenase (Boehringer Mannheim), and incubated 1.5 h at 37°C on a rocker. The resultant cell suspension was
15 filtered sequentially through 100 mm and 40 mm nylon mesh filters and then washed 2' in RPMI 1640 supplemented with 25 mM HEPES and 10% FCS.

In vitro cell stimulation and cytokine assay

Isolated cells were stimulated in RPMI 1640 supplemented with 10% FCS, 5%
20 NCTC (M. A. Whittaker Bioproducts), 1% penicillin/streptomycin/0.25% fungisozone (GibcoBRL, Gaithersburg, MD), 2 mM glutamine (GibcoBRL), 5×10^5 M ME (Sigma Chemical Co., St. Louis, MO), and 15 mM HEPES at a density of 1×10^6 cells per 1.0 ml final volume. Cells were cultured in 24-well tissue culture plates (Costar, Cambridge, MA) in the presence of plate-bound anti-CD3 (10 μ g/ml; Pharmingen) with
25 soluble anti-CD28 (1 μ g/ml; Pharmingen) and IL-2 (50 U/ml; Chiron, San Diego, CA) at 37°C in humidified 6% CO₂. Culture supernatants were harvested after 48 h and frozen at -20°C until assayed. IFN- γ production in culture supernatants was assayed by ELISA using the Pharmingen protocol in association with coating, standard, and
30 biotinylated secondary Abs obtained from Pharmingen and HRP-streptavidin obtained from Zymed (South San Francisco, CA).

Flow cytometry

To characterize T cell phenotypes in recovered LPL, IEL and splenocytes, flow cytometric analysis with Abs to murine cell surface determinants (Pharmingen) was employed. For this purpose, cells were washed 2x in FACS buffer (NIH Media Unit),
 5 resuspended at 10^7 cells/ml in FACS buffer, and transferred to FACS tubes (Becton Dickinson, Mountain View, CA). To prevent nonspecific FcR-mediated binding of Abs, 50-100 $\mu\text{g/ml}$ of anti-Fc ϵ R (2.4G2; Pharmingen) was added to each tube 3 min prior to staining. Cell suspensions were stained with 1 $\mu\text{g/ml}$ for 30 min on ice with FITC-conjugated CD3, FITC-conjugated CD4, PE-conjugated CD8, PE-conjugated
 10 $\alpha_4\beta_7$, biotin-conjugated ICAM-1 (CD54), or biotin-conjugated CD69. Cells stained with biotin-conjugated Abs were subsequently washed once in FACS buffer and incubated on ice for 30 min with streptavidin-PE at 5 $\mu\text{g/ml}$. All stained cells were washed 2x in FACS buffer, resuspended at 1×10^6 cells/ml, and examined on a FACSCAN analyzer, Lysis II software (Becton Dickinson). Nonviable cells were
 15 excluded by forward angle scatter or by propidium iodide uptake.

Statistical analysis

Descriptive statistics and testing for significance of differences were assessed by Student's *t* test using the StatWorks and Microsoft Excel statistical analysis
 20 computer programs.

Treatment of TNP-OVA induced colitis by either anti- $\alpha^E\beta_7$ or anti- $\alpha_4\beta_7$ mAb is associated with reduction of LPL and IEL cell numbers, but increase of splenic cell numbers.

25 To evaluate the role of $\alpha^E\beta_7$ vs. $\alpha_4\beta_7$ in a newly developed TNP-OVA/IL-2-/- model of colitis, IL-2-/- mice maintained under specific pathogen-free condition were immunized with TNP-OVA and given either anti- $\alpha^E\beta_7$ or anti- $\alpha_4\beta_7$ mAbs at the same time of immunization, as described above. Therefore, colitis was induced in IL-2-/- mice that were maintained in specific pathogen-free environment and evaluated the
 30 affect of concomitant injection of $\alpha^E\beta_7$ mAb. As shown in Figure 1, severe colitis was seen in mice injected with TNP-OVA, however, when TNP-OVA was co-injected with

anti- $\alpha^E\beta_7$ mAb the colitis was prevented and microscopic examination of the colonic mucosa resembled the one seen in PBS-injected mice. This was reflected by significant reduction in colitis severity score (CSS); IL-2^{-/-} mice injected with TNP-OVA, CSS: 3-4 vs. TNP-OVA + anti- $\alpha^E\beta_7$ injected, CSS: 0-2 (Table1). Furthermore, this inhibition of colitis was associated with a fourfold reduction in LPL numbers and twofold reduction in IEL numbers in mice that were co-injected with $\alpha^E\beta_7$ mAb (IL-2^{-/-} LPL: TNP-OVA; $180 \pm 5 \times 10^6$ vs. TNP-OVA co-injected with anti- $\alpha^E\beta_7$ $6.5 \pm 0.6 \times 10^6$. IL-2^{-/-} IEL: TNP-OVA; $5.2 \pm 1.8 \times 10^6$ vs. TNP-OVA co-injected with anti- $\alpha^E\beta_7$; $2.4 \pm 0.9 \times 10^6$). Interestingly, when the total number of cells were looked at within the spleen there was a slight increase of total cell numbers (Table1). Similarly, TNP-OVA-induced colitis was significantly reduced when mice with established colitis were treated with two anti- $\alpha^E\beta_7$ mAb injections, TNP-OVA CSS: 3-4 vs. TNP-OVA + anti- $\alpha^E\beta_7$ mAb injections CSS: 1-2. To evaluate the role of $\alpha_4\beta_7$ in this model of colitis, IL-2^{-/-} mice were co-injected with TNP-OVA and anti- $\alpha_4\beta_7$. As shown in Table 1, injection of anti- $\alpha^E\beta_7$ also prevented the development of TNP-OVA-induced colitis in IL-2^{-/-} mice. Therefore, the above data demonstrates that not only is the injection of anti- $\alpha^E\beta_7$ mAb capable of preventing TNP-OVA-induced colitis in IL-2^{-/-} mice, but can also directly ameliorate established colitis.

20 *Administration of anti- $\alpha^E\beta_7$ mAb in to IL-2^{-/-} mice reduces the number of infiltrating CD4⁺ T cells within the LPL, but increases CD4⁺ T cells within the spleen.*

It has previously been demonstrated that CD4⁺ T cells are the principal effector cells involved in the pathogenesis of TNP-OVA-induced colitis in IL-2^{-/-} mice(12, 17). Therefore it is interesting to see if the administration of anti- $\alpha^E\beta_7$ vs. anti- $\alpha_4\beta_7$ had different effects on lymphoid organ compartmentalization of CD4⁺ and CD8⁺ T cells. As shown in Table 1, injection of anti- $\alpha^E\beta_7$ mAb was most associated with reduction of total number of cells within the LP compartment. This reflects previous observations (and demonstrated here in Table 1 and figure 1) that the most severe inflammation is found within the LP of the colon. Furthermore, as shown in figure 2, anti- $\alpha^E\beta_7$ mAb injection led to a five-fold reduction of LP CD4⁺ lymphocytes; total number of CD4⁺ LPL in TNP-OVA vs. TNP-OVA + anti- $\alpha^E\beta_7$, 21.7×10^6 and 4.7×10^6 , respectively.

Within the IEL compartment anti-IEL treatment was associated with modest reduction of CD8+ T cells; TNP-OVA: 4×10^6 vs. TNP-OVA + anti- $\alpha^E\beta_7$: 1.6×10^6 (figure 3). Interestingly, the total number of CD4+ T cells within the IE compartment was not significantly altered by the injection of anti- $\alpha^E\beta_7$ mAb into IL-2-/- mice. Furthermore, 5 when we analyzed similarly treated IL-2+/+ mice no changes in the total number of CD4+ T cells were observed within any of the compartments evaluated (Table 1).

To further evaluate if the reduction of CD4+ lymphocytes in the LP of TNP-OVA immunized IL-2-/- mice following anti- $\alpha^E\beta_7$ mAb treatment was due to the 10 deletion of $\alpha^E\beta_7$ + lymphocytes or rather an effect on lymphocyte distribution, the number of T cells within the peripheral lymphoid organs was also evaluated. As shown in figure 4, injection of anti- $\alpha^E\beta_7$ was associated with significantly increased numbers of CD4+ T cells within the spleen; TNP-OVA: 13.5×10^6 vs. TNP-OVA + anti- $\alpha^E\beta_7$: 32.4×10^6 . Within the mesenteric lymph node compartment total CD4+ T cell numbers 15 were similar in TNP-OVA immunized IL-2-/- mice whether or not they had received anti- $\alpha^E\beta_7$ or not.

Administration of $\alpha^E\beta_7$ mAb results in decreased IFN- γ production within LPL but increased IFN- γ production within the Spleen

20 Since it is now well established that the severity of chronic mucosal inflammation correlates with the level of IFN- γ production by inflammatory CD4+ T cells in many murine models of colitis whether the treatment with anti- $\alpha^E\beta_7$ mAb affects IFN- γ levels was further analyzed. Thus, in initial studies the IFN- γ production of LPL isolated from treated mice were compared with LPL isolated from untreated 25 mice. After isolation, LPL were directly stimulated *in vitro* with anti-CD3 and anti-CD28 mAbs and IFN- γ production in the culture supernatants was analyzed. As demonstrated in figure 5, T cells from anti- $\alpha^E\beta_7$ mAb treated IL-2-/- mice manifested a five-fold reduction of IFN- γ when compared to untreated TNP-OVA immunized mice: TNP-OVA + anti- $\alpha^E\beta_7$ mAb: IFN- γ 46 ± 15 U/ml vs. IFN- γ 225 ± 72 U/ml ($p = 0.003$). 30 In a similar series of studies, splenic T cell IFN- γ production from treated vs. untreated IL-2-/- mice was evaluated. As shown in figure 5, TNP-OVA immunization alone

induced a three-fold increase in IFN- γ production in the splenic T cells when compared to PBS injected control IL-2-/- mice. Furthermore, this amount of splenic IFN- γ secretion was even higher, following co-injection of TNP-OVA and anti- $\alpha^E\beta_7$; TNP-OVA alone: IFN- γ 97 ± 20 U/ml vs. TNP-OVA + anti- $\alpha^E\beta_7$ mAb: IFN- γ 202 ± 14 U/ml, $p = 0.005$. Of note, in none of the above conditions could we find a significant IL-4 production.

Above data indicate that administration of anti- $\alpha^E\beta_7$ mAb is preventing the influx and/or retention of T cells that are committed to the Th1 cytokine pathway into the colonic LP.

Anti- $\alpha^E\beta_7$ mAb treatment prevents accumulation of CD3+/CD54+ LPL in TNP-OVA immunized IL-2-/- mice.

The expression of adhesion molecules is undoubtedly an important regulatory element during the induction of chronic mucosal inflammation. ICAM-1 (CD54) is one member of the Immunoglobulin superfamily that is significantly up-regulated on T cells during Th1-mediated inflammation and the binding to its ligand VLA-1 has been demonstrated not only to enhance T cell adhesion but also to induce intracellular T cell activation (27-32). Furthermore, it has previously been shown that one of the characteristics of the TNP-OVA-induced inflammatory response in IL-2-/- mice is the induction of CD54 and CD69 (21, 26). Since anti- $\alpha^E\beta_7$ mAb treatment lead only to decreased IFN- γ production within the LP compartment but not in the spleen we were interested to see if this was reflected by the expression of the above described T cell activation markers. As demonstrated in Table 2, there was a significant decrease of CD3+/CD54+ LPL following co-injection of TNP-OVA and anti- $\alpha^E\beta_7$ compared to TNP-OVA alone, 16.4×10^6 and 2.6×10^6 , respectively, and this was also evident by fewer number of LPL expressing CD54 following anti- $\alpha^E\beta_7$ treatment. However, when the T cell expression of CD54 within the spleen was evaluated there was a notable increase of CD3+/CD54+ lymphocytes following anti- $\alpha^E\beta_7$ treatment; total number of CD3+/CD54+ lymphocytes following co-injection of TNP-OVA and anti- $\alpha^E\beta_7$ mAb

165.6 x 10⁶ vs. TNP-OVA alone 135 x 10⁶. Similar findings were seen for the expression of another, but less characterized, activation marker, CD69 (Table 2).

Although the present process has been described with reference to specific
5 details of certain embodiments thereof, it is not intended that such details should be regarded as limitations upon the scope of the invention except as and to the extent that they are included in the accompanying claims.

Throughout this application, various publications are referenced. The
10 disclosures of these publications in their entireties, as well as the references cited in these publications, are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

Table 1. $\alpha^E\beta_7$ prevents TNP-OVA induced colitis in IL-2^{-/-} mice

		Spleen Cell no. $\times 10^6$	LPL Cell no. $\times 10^6$	IEL Cell no. $\times 10^6$	Colitis Severity Score	n
<u>IL-2^{-/-}</u>	PBS	40 \pm 0.8	3.6 \pm 2.1	1.7 \pm 1.1	0-1	3
	TNP-OVA	150 \pm 4	24.9 \pm 10	5.2 \pm 1.8	3-4	4
	TNP-OVA +					4
	anti- $\alpha^E\beta_7$	180 \pm 5	6.5 \pm 2.6	2.4 \pm 0.9	0-2	
	TNP-OVA +					3
	anti- $\alpha_4\beta_7$	178 \pm 9	7.1 \pm 3.5	2.0 \pm 1.1	0-2	
<u>IL-2^{+/+}</u>	PBS	6.8 \pm 0.6	3.1 \pm 0.5	2.2 \pm 0.9	0	4
	TNP-OVA	8.2 \pm 1.1	3.5 \pm 0.8	1.9 \pm 1.2	0	4

Table 2. T cells activation following anti- $\alpha^E\beta_7$ administration

	% Positive	Spleen CD3+		LPL CD3+		IEL CD3+	
		CD54	CD69	CD54	CD69	CD54	CD69
<u>IL-2-/-</u>	PBS	38±6	35±3	25±5	29±5	6	n.d.
	TNP-OVA	90±3	70±1	66±7	71±5	74±8	n.d.
	TNP-OVA	92±4	66±4	40±5	60±2	64±9	n.d.
	+ anti- $\alpha^E\beta_7$						
<u>IL-2+/+</u>	PBS	28±4	n.d.	20±3	n.d.	33±6	n.d.
	TNP-OVA	31±6	n.d.	28±4	n.d.	35±3	n.d.

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What is claimed is:

1. A method of treating inflammation in a subject, comprising administering to the subject an antagonist to $\alpha E\beta 7$.
2. The method of claim 1, wherein the antagonist is an antibody.
3. The method of claim 2, wherein the antibody is an $\alpha E\beta 7$ Mab.
4. The method of claim 1, wherein the inflammation is associated with an inflammatory bowel disease.
5. The method of claim 1, wherein the inflammation is associated with asthma.
6. The method of claim 1, wherein the inflammation is associated with rheumatoid arthritis.
7. The method of claim 1, wherein the inflammation is associated with autoimmune diseases.
8. The method of claim 1, wherein the inflammation is associated with allergies.
9. The method of claim 1, wherein the inflammation is associated with transplant rejection.

10. The method of claim 1 wherein the inflammation is associated with graft vs. host diseases.
11. A method of preventing inflammation in a subject, comprising, administering to the subject an antagonist to $\alpha E\beta 7$.
12. The method of claim 11, wherein the antagonist is an antibody.
13. The method of claim 12, wherein the antibody is an $\alpha E\beta 7$ Mab.
14. The method of claim 11, wherein the inflammation is associated with an inflammatory bowel disease.
15. The method of claim 11, wherein the inflammation is associated with asthma.
16. The method of claim 11, wherein the inflammation is associated with rheumatoid arthritis.
17. The method of claim 11, wherein the inflammation is associated with autoimmune diseases.
18. The method of claim 11, wherein the inflammation is associated with allergies.
19. The method of claim 11, wherein the inflammation is associated with transplant rejection.

20. The method of claim 11, wherein the inflammation is associated with graft vs. host diseases.
21. The method of claim 1, further comprising administering another therapeutic agent.
22. The method of claim 21, wherein the other therapeutic agent is an antibody.
23. The method of claim 22, wherein the antibody is an $\alpha 4\beta 7$ Mab.
24. The method of claim 21, wherein the other therapeutic agent is a cytokine.
25. The method of claim 21, wherein the other therapeutic agent is an immunomodulatory agent.
26. The method of claim 11, further comprising administering another therapeutic agent.
27. The method of claim 26, wherein the other therapeutic agent is an antibody.
28. The method of claim 27, wherein the antibody is an $\alpha 4\beta 7$ Mab.
29. The method of claim 26, wherein the other therapeutic agent is a cytokine.
30. The method of claim 26, wherein the other therapeutic agent is an immunomodulatory agent.

31. A method of screening for a substance effective in reducing the inflammatory effects of $\alpha E\beta 7$, comprising:
- a) administering the substance to an animal having an established inflammatory disease;
 - b) assaying inflammatory tissue cells from the animal for an amount of secretion of proinflammatory cytokines, inflammatory cytokines or chemokines, whereby a decrease in the amount of proinflammatory cytokines, inflammatory cytokines or chemokines secreted by the inflammatory tissue cells of the animal as compared to the amount of proinflammatory cytokines, inflammatory cytokines or chemokines secreted by inflammatory tissue cells of a control animal having an inflammatory disease and without having the substance administered indicates the substance is effective in reducing the inflammatory effect of $\alpha E\beta 7$;
 - c) evaluating the effect of the antagonist of $\alpha E\beta 7$ in reducing the adhesion/retention/influx of $\alpha E\beta 7$ + inflammatory cells into the inflammatory tissue, a reduction indicating that the antagonist is acting at the level of $\alpha E\beta 7$.
32. The method of claim 31 wherein the animal has an established inflammatory disease produced by a hapten reagent.
33. The method of claim 32 wherein the hapten reagent is a 2,4,6 trinitrophenol (TNP) conjugate of keyhole limpet hemocyanin (KLH).
34. The method of claim 33 wherein the animal is a mouse.

35. A method of screening for a substance effective in preventing the inflammatory effects of $\alpha E\beta 7$, comprising:
- administering the substance to an animal susceptible to an inflammatory disease;
 - subjecting the animal to treatment that will induce an inflammatory response; and
 - assaying inflammatory tissue cells from the animal for an amount of secretion of proinflammatory cytokines, inflammatory cytokines or chemokines, whereby a lack of increase in the amount of proinflammatory cytokines, inflammatory cytokines or chemokines secreted by the inflammatory tissue cells of the animal as compared to an increase in the amount of proinflammatory cytokines, inflammatory cytokines or chemokines secreted by inflammatory tissue cells of a control animal having an inflammatory disease and without having the substance administered indicates the substance is effective in preventing the inflammatory disease by inhibiting the inflammatory effect of $\alpha^E\beta 7$,
 - evaluating the effect of the antagonist of $\alpha E\beta 7$ in reducing the adhesion/retention/influx of $\alpha E\beta 7+$ inflammatory cells into the inflammatory tissue.
36. The method of claim 35, wherein the animal is a mouse.
37. The method of claim 36, wherein the treatment that will induce the inflammatory response is the introduction of an effective amount of a hapten reagent.

38. The method of claim 37, wherein the hapten reagent is a 2,4,6 trinitrophenol(TNP) conjugate of limpet hemocyanin (KLH).
39. The method of claim 31, wherein the cytokine is IFN- γ
40. The method of claim 35, wherein the cytokine is IFN- γ .
41. A composition comprising an α E β 7 antagonist and a pharmaceutically acceptable carrier, wherein the antagonist is not an anti- α E β 7 antibody.
42. A composition comprising an α E β 7 antibody and a pharmaceutically acceptable carrier, wherein the carrier is not hybridoma supernatant.
43. A composition comprising an α E β 7 antibody, a second anti-inflammatory agent and a pharmaceutically acceptable carrier.

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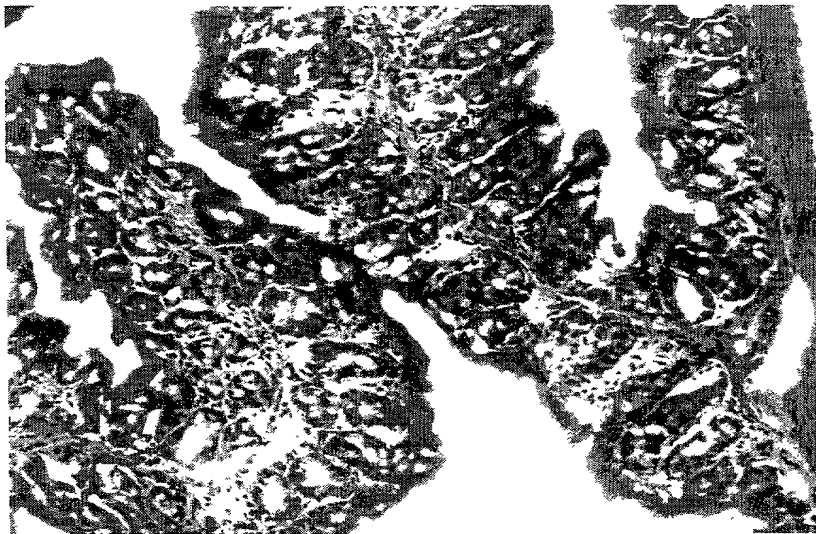


FIG.1A



FIG.1B

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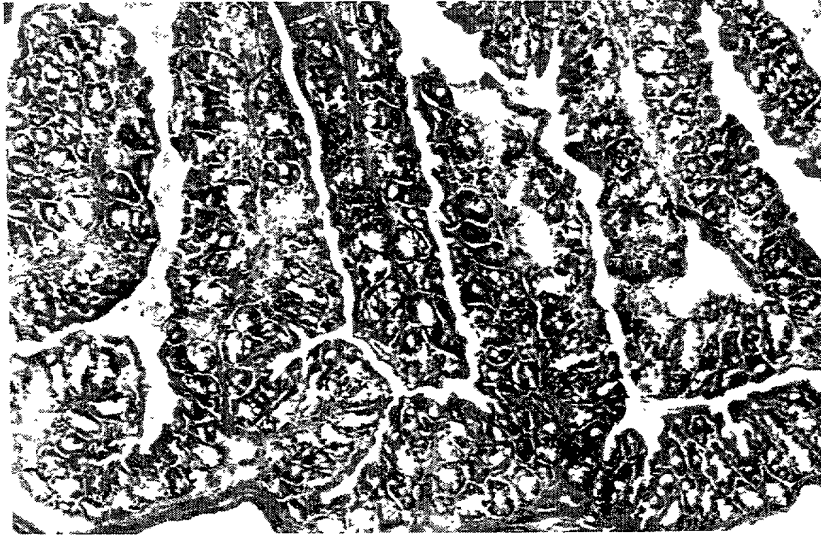


FIG.1C



FIG.1D

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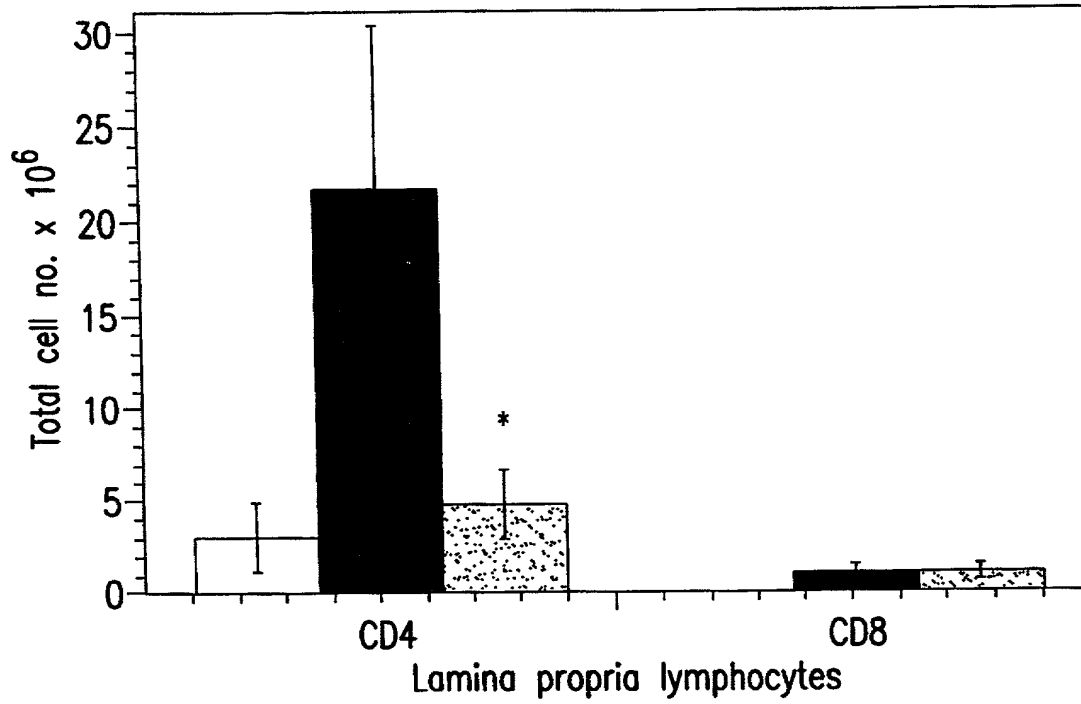


FIG.2

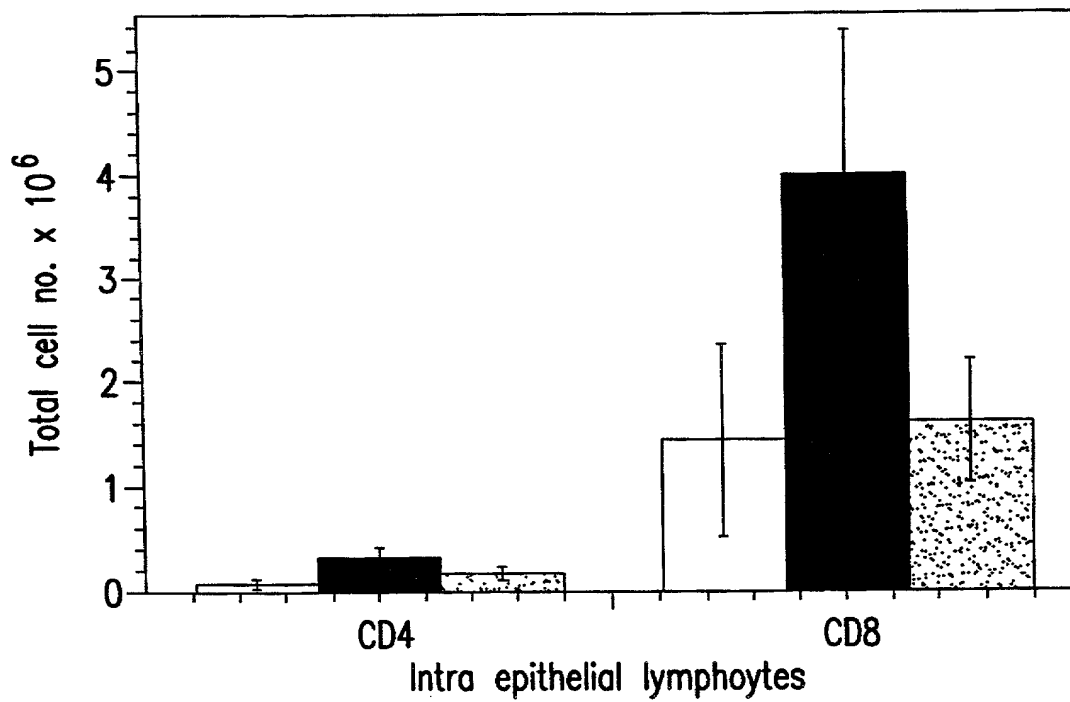


FIG.3

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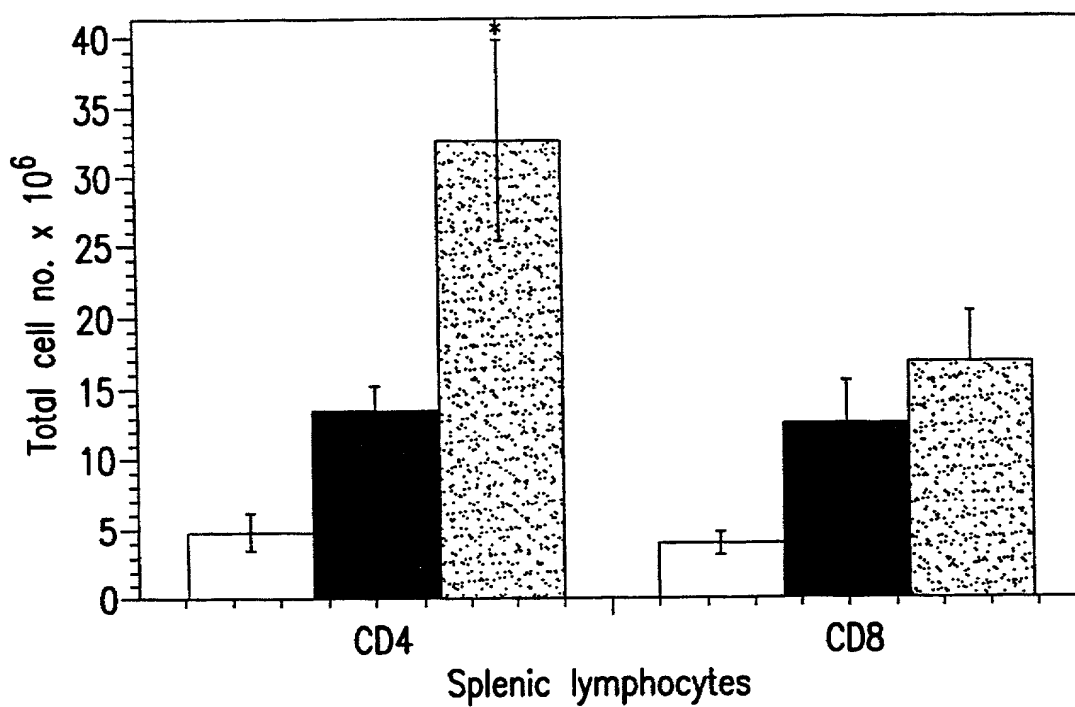


FIG.4

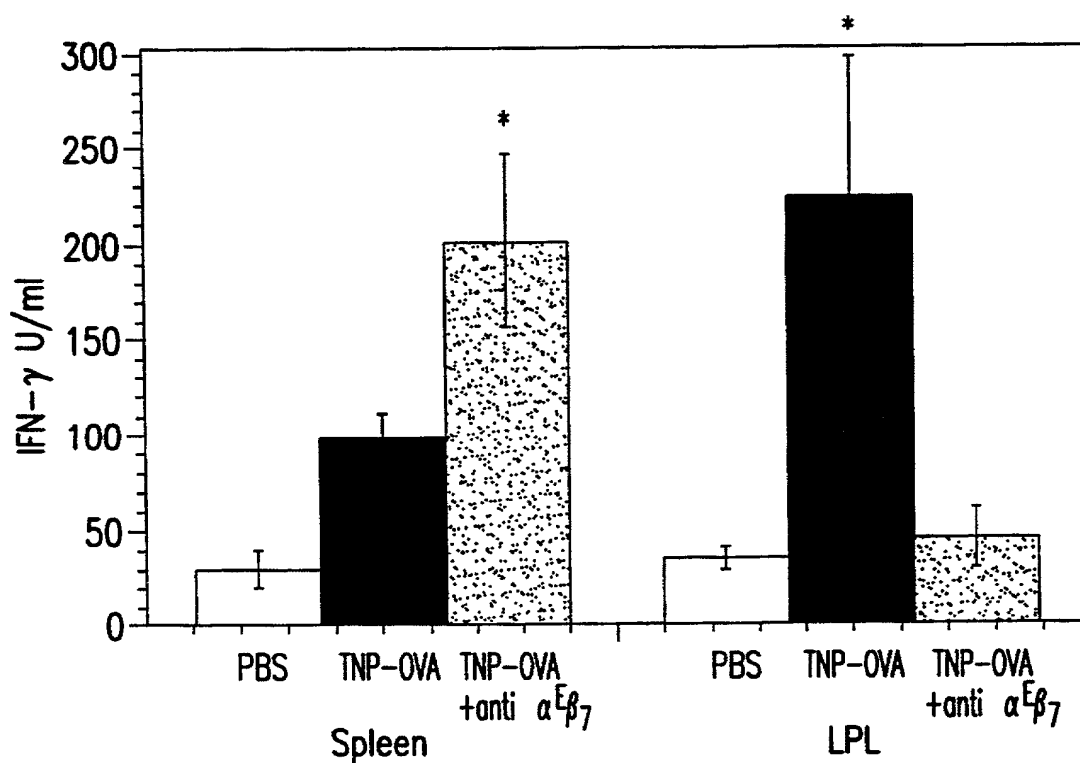


FIG.5

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

(X) Original () Supplemental () Substitute () PCT

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled "**ANTAGONISTS OF THE ALPHA EPSILON BETA 7 INTEGRIN AS THERAPEUTIC AGENTS FOR INFLAMMATORY DISEASES,**" which is described and claimed in the specification

(check one) ☐ which is attached hereto, or
 ☒ which was filed on May 23, 2001, as Application Serial No. 09/856,544 and with amendments through (if applicable), or
 ☐ in International Application No., filed , and as amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information known by me to be material to the patentability of the claims of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code §119 (a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATIONS: (ENTER BELOW IF APPLICABLE)			PRIORITY CLAIMED (MARK APPROPRIATE BOX BELOW)	
APP. NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	YES	NO
PCT/US99/27817	PCT	23 November 1999	X	

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

APPLICATION NUMBER	FILING DATE
60/109,957	25 November 1998 ✓

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information known by me to be material to the patentability of the claims of this application as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS (MARK APPROPRIATE COLUMN BELOW)		
		PATENTED	PENDING	ABANDONED

I hereby appoint the following attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

James C. Haight, Reg. No. 25,588, Robert Benson, 33,612, Jack Spiegel, 34,477, Susan S. Rucker, 35,762, David R. Sadowski, 32,808, Steve Ferguson, 38,448; John Peter Kim, 38,514, Stephen L. Finley, 36,357, Norbert Pontzer, 40,777, Richard U. Rodriguez, 45,980, and Marlene Shinn, 46,005.

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Atlanta, Georgia 30303-1811

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Inventor's signature: *Biorn R. Ludviksson*

Date: 10/2/01

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Citizenship: Iceland ✓

Full name of second inventor: WARREN STROBER

Inventor's signature: _____

Date: _____

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Full name of third inventor: ROLF O. EHRHARDT

Inventor's signature: _____

Date: _____

Residence: 1676 Sanchez Street, San Francisco, California 94114

Post Office Address: 1676 Sanchez Street, San Francisco, California 94114

Citizenship: United States

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

(X) Original () Supplemental () Substitute () PCT

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled "**ANTAGONISTS OF THE ALPHA EPSILON BETA 7 INTEGRIN AS THERAPEUTIC AGENTS FOR INFLAMMATORY DISEASES,**" which is described and claimed in the specification

(check one) ☐ which is attached hereto, or
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PRIOR FOREIGN APPLICATIONS: (ENTER BELOW IF APPLICABLE)			PRIORITY CLAIMED (MARK APPROPRIATE BOX BELOW)	
APP. NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	YES	NO
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Full name of first inventor: BJORN R. LUDVIKSSON

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Inventor's signature: Warren Strober Date: 9/3/01

Residence: 8301 Whittier Boulevard, Bethesda, Maryland 20817 MD

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3-00 Full name of third inventor: ROLF O. EHRHARDT

Inventor's signature: R. Ehrhardt Date: 9/2/01

Residence: 1676 Sanchez Street, San Francisco, California 94114 CA

Post Office Address: 1676 Sanchez Street, San Francisco, California 94114

Citizenship: United States ✓